SUMMARY AND CONCLUSIONS

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Since the tenability of the theory of neurobiotaxis was questioned (William *et al.*, 1989) in Gray's text book of anatomy, it was decided to study the facial nucleus in the human foetus and adult. Moreover the information regarding the nucleus in the human foetus is limited. The work was extended to *Macaca radiata* (bonnet monkey) the most commonly used primate for experimental work in Tamil Nadu because we did not find any record of such work in the available literature.

Since facial nerve is the most commonly affected cranial nerve (McGovern *et al.*, 1966) and the effects of facial nerve injury forms 43% of the cause for facial paralysis (Bento et. al. 1985 ~~and Miniti 1989~~) and according to Brodal (1981) there is no differences between experimentally produced lesions and those which occur in human cases, the bonnet monkey was chosen for experimental work.

The facial nucleus in ten human foetuses ranging from 8 to 36 weeks, three human adults and five bonnet monkeys - a foetus (near full term), a newborn and three adults were used for the study.

The intra and extra cranial course of the facial nerve, its extracranial branches and the muscles innervated by it were dissected in ten adult *Macaca radiata*.

The position of the facial nucleus in two adult monkeys was delineated by the use of the tracer, DiI.

Transections of various parts or whole facial nerve, unilaterally, just before it entered the parotid gland were made in ten adult bonnet monkeys and the effect on the facial nucleus, the proximal and distal parts of the nerves and on the muscles supplied were noted from paraffin wax sections stained with neuro histologic stains. Three adult monkeys were used as controls.

The migration of cells, position, length, subdivisions and neuronal characteristics of the normal nucleus in human and monkey material were studied on paraffin wax sections stained with cresyl fast violet, luxol fast blue, Golgi and fuchsin.

Morphometric analysis of the neuronal number, volume and surface area per mm^3 of the facial nucleus in the human foetus and in the subdivisions of the monkey facial nucleus (normal and experimental) was done.

Analysis of variance was applied to study the morphometric results and where differences were found, Duncan's multiple range test was applied and their levels of significance calculated.

Migration of the neurons of the human foetal facial nucleus was incomplete at 8 weeks of development. The facial nuclear complex could not be delineated at its caudal levels from the abducens nucleus at 8 weeks. But it was a separate entity by 12 weeks.

The craniocaudal length of the nucleus increased from 0.66 to 2.5 mm from 8 to 36 weeks. A spurt in the length of the facial nucleus in the human foetus was seen between the 28th to 36th week of development.

The angle of orientation of the facial nucleus, showed an increase of 50 degrees between the 8th and 36th week of intra uterine life in the human. Of this, 17 degrees were between the 16th to 20th weeks, which is more than at any other stage of development. The ventrolateral shift of the facial nucleus brought it close to the spinal nucleus and tract of the trigeminal.

Graphic reconstruction of the cranial nuclei in the lower pons of the human foetuses showed the relative positions of the nuclei from the 8th to 36th week of intra uterine life. Upto 20 weeks, the abducent nucleus was at the caudal end of the facial nucleus. In the 32 weeks foetus, it was about the middle of the facial nuclear complex and at 36 weeks, only a very small cranial part of the facial nucleus was superior to the abducent nucleus. In the adult human, however, the abducent nucleus was cranial to the facial nucleus.

The facial nucleus in the human consists of motoneurons and some fusiform cells. The nucleus is made up of subdivisions which can be made out by the difference in the distribution and size and types of cell bodies, although the processes are not confined to the subdivisions.

In the human foetus, the subdivisions of the nuclear complex into dorsal, medial, intermediate, ventromedial, ventrolateral and lateral was distinct from

the 28th week of gestation. In the human adult also the same subdivisions could be seen. No attempt was made to delineate the accessory facial nucleus.

The neuronal number, volume and surface area per mm^3 showed a general decrease in values from 8 weeks to 32 or 36 weeks. But the decrease was not steady and a sudden temporary increase in the values of all three parameters occurred between 16 to 20 weeks. The sudden increase in the number was seen at 16 weeks, but it was not statistically significant. The increase of volume was at 18 weeks and it was more than the volume at 8 weeks and was statistically significant. The increase in surface area per mm^3 at 20 weeks was a significant deviation from the 18 weeks and 24 weeks foetuses.

The mean neuronal diameter increased from 15.3μ at 8 weeks to 30.8μ at 36 weeks of development.

Myelination gliosis which was first seen at 14 weeks of development became clear by 16 weeks and myelinated nerves were seen by 28 weeks.

The facial nucleus in *M.radiata* also shifted ventrolaterally as seen in the sections through the foetal, newborn and adult material.

The length of the nucleus in the adult monkeys was in direct correlation with the weight of the monkeys.

The subdivisions of the nucleus were dorsal, dorsolateral, intermediate, ventral and ventromedial. In all three stages studied the intermediate nucleus showed the largest neurons and the smallest were in the ventromedial nucleus.

The overall difference in neuronal number, volume and surface area per mm^3 in the nucleus of the foetus when compared with the newborn and adult was statistically highly significant ($p<0.01$). But a similar comparison between the newborn and adult was not significant.

The adult monkey nucleus showed multipolar and fusiform cells. Neuronal processes were seen close to blood vessels.

The intraneural and intracranial course of the facial nerve was nearly similar to that in the human, but the nervus intermedius and facial nerve proper formed a single nerve.

After emerging from the stylomastoid foramen it gave off branches to the stylohyoid and posterior belly of the digastric muscles and also the posterior auricular branch, before entering the parotid gland. At its entry, it divided into an upper temporofacial and a lower cervicofacial divisions. The upper trunk supplied the frontalis, auricularis anterior and the orbital musculature through the temporal branches; and the zygomatic muscle mass and adjacent parts of the orbicularis oculi and nasal muscles through its zygomatic branches. The lower trunk supplied the other facial muscles and the platysma through its buccal, mandibular and cervical branches.

Implantation of the tracer DiI into the facial nerve of a fixed monkey and injection into a live animal showed the position of the nucleus and the neuronal grouping.

Unilateral transection of the nerve at its entry into the parotid gland caused paralysis of the muscles of the ipsilateral side of the face. When only the upper or lower trunks were unilaterally transected only the upper or lower part of the face respectively was affected. All the monkeys exhibited postural imbalance but it disappeared in monkeys which were allowed to survive for more than two months. The apparent movements which appeared by the 14th day did not progress.

Observations on the facial nucleus was made from the 2nd to 180 post operative days. The affected cells were scattered throughout the nucleus. The changes within the first two weeks were vasodilation, neuronal swelling, adhesion, glial reaction at synaptic terminals, invasion by leucocytes, microglial activation, chromatolysis, neuronal loss and glial aggregations and also some neuronal aggregations.

On the 14th post operative day some restitution processes were seen but degenerative processes also continued and by the 21st day loss of normal neuronal grouping pattern and glial fibres in the areas of neuronal loss were seen. But some normal neurons were still seen.

Remarkable cell shrinkage and destruction was seen by 60 days. At 120 and 180 days along with atrophied cells, some reorganisation of large neurons and their fibres were seen. By 120 days vascular reorganisation was complete.

Changes such as neuronal adhesion, chromatolysis and increase in vascularity were seen in the contralateral nucleus also. But these were few

compared to those on the operated side. The intraneural part of the transected facial nerve showed degeneration from the 14th day onwards and by 180 days there was considerable reduction in the number of nerve fibres.

The distal part of the transected nerve showed loss of nerve fibres and their sheaths.

The facial muscles of the monkey which were allowed to survive for 180 days showed fibrosis and atrophy. The neuromuscular junctions were destroyed.

The observations on the normal material (human and monkey) and results of the experimental work were assessed in relation to the available literature and the following conclusions were drawn.

Since clusters of cells at the caudal end of the nucleus had not separated from the lateral margin of the abducent nucleus in the 8 weeks human specimen, but was a separate entity by 12 weeks, the migration was completed between the 8th and 12th weeks, perhaps at 10½ weeks as stated by Jacobs (1971).

The 50° lateral shift in the orientation of the facial nucleus from 8 to 36 weeks of intrauterine human development, along with a ventral shift in position brought the facial nucleus to lie close to the spinal tract and nucleus of the trigeminal nerve. Thus the migration of the cells from near the abducent

nucleus ventrolaterally towards the trigeminal spinal tract and nucleus would favour the theory of neurobiotaxis.

Graphic reconstruction of the cranial nuclei in the lower pons of human foetuses showed that the positions of the abducent and facial nuclei varied at different stages of development, while the facial nucleus was seen to be marginally or entirely rostral to the abducent nucleus till the 20th week, only its rostral most part was superior to the abducent nucleus at 36 weeks of development. In the 20 year old adult, however, the facial nucleus was caudal to the abducent nucleus. So changes occur after birth and perhaps through early childhood also.

While Jacobs (1971) noticed two spurts during growth in length of the human foetal facial nucleus, one between 10 and 11 weeks and the other between 21 weeks and birth, we found only one such spurt and it was between 28 and 36 weeks. This spurt correlated with the increase in the size of the cells, neuropil and myelination.

In the adult monkeys there was a direct correlation between the length of the nucleus and the weight of the monkeys. Since we had not weighed the foetuses we do not know if such a relationship exists in the human foetuses. The nuclear length is not dependent on the CR length.

Sub-divisions of the facial nucleus in the human foetus were distinguishable only from the 28th week and not from the 21st week as reported by Nara *et al.* (1989).

The extension of dendritic processes beyond the morphologic subdivisions in the human adult may be attributed to functional coordination as suggested by Welt and Abbs (1990) who found a similar pattern in *M.fascicularis*.

The positions of the subdivisions in the *M.radiata* are different from that described by Satoda *et al.* (1987) in *M.fuscata*.

The cells in the adult *M.radiata* ranged from 12.3 to 48.5 μ and so are slightly larger than in *M.fascicularis* (Wett & Abbs 1990) and may reflect the absence of gamma motoneurons.

The neuronal diameter of 31.68 μ in the adult monkey was close to that of the 36 week old human foetus (30.75 μ).

The statistically significant increase in the volume/mm^3 at 18 weeks and the significant deviation in the surface area/mm^3 at 20 weeks in the human foetus supports the conclusion drawn by Bhargava, Kamakshi and Dodge (1976) from their study on human placenta, that bulk or volume is laid down before the area becomes significant.

In *M. radiata*, the overall highly significant difference in neuronal number, volume and surface area/mm^3 in the foetal nucleus when compared with the new born and adult monkeys while there is no significant difference between the newborn and adult monkeys would mean that the increase in the length of the nucleus seen between the newborn and adult must be due to the increase in the neuropil and myelination after birth. The slight increase in the

surface area/mm^3 from neonate to adult may be a reflection of the spreading of neuronal processes from their early compact arrangement to the adult stage.

Myelination gliosis was seen from 14 weeks in the human foetuses and myelination could be seen clearly from the 28th week onwards.

The intracranial course of the facial nerve in *M.radiata* was similar to that described by Gasser and Hendrickx (1977) in the baboon and nearly similar to that in man.

The pattern of branching of the facial nerve after its exit from the stylomastoid foramen was different from that in the Japanese monkey (Satoda *et al.*, 1987) and in *M.mulatta* (Huber, 1933).

The position and organization of the facial nucleus as defined by the tracer DiI was not different from that seen in histological sections.

Partial and total unilateral transections of the facial nerve just before it entered the parotid gland resulted in the facial nuclei of both sides showing changes, although the changes were few on the contralateral side . The facial muscles on the contralateral side showed no changes.

On the ipsilateral side, the cells showing changes were scattered throughout the nucleus in all the subdivisions even if only a part of the nerve was transected. So, as in the dorsal root ganglia (Jacob, 1966) the cells of origin of the nerve fibres in the branches of the facial nerve are not located in distinct groups within the facial nucleus.

The changes found within the facial nucleus between the 2nd and 14th post operative days, i.e., vasodilation, infiltration by leucocytes and other vascular elements, microglial activation, glial aggregation and chromatolytic changes in the neurons and the changes in the proximal and distal parts of the nerve and the facial muscles are comparable to those reported by other workers.

The neuronal aggregations which were seen by the 2nd post operative day was interesting because such groups seemed to survive while the scattered neurons disappeared. The groups of neurons seen even 6 months after transection of the nerve must be those whose axons formed the branches given off before the nerve entered the parotid gland.

The 14th post operative day seemed to be an important one because some restitutional processes occurred in cells along with atrophy of many other neurons. Moreover, slight apparent movements of the face were seen at this time. The intraneural part of the transected nerve showed degenerative changes after the 14th day. So it seems to reinforce the view that any reconstructive surgery undertaken should be done before the 14th day if it is not possible to do it sooner.